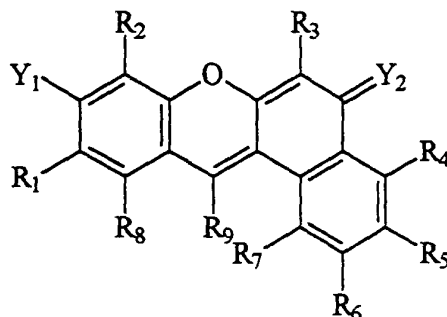


WE CLAIM:

1. An asymmetric benzoxanthene dye compound having the formula:

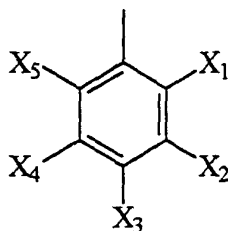


5 wherein:

Y₁ and Y₂ taken separately are selected from the group consisting of hydroxyl, oxygen, imminium, and amine;

R₁-R₈ taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, sulfone, amino, imminium, amido,
10 nitrile, lower alkoxy, linking group, and combinations thereof; and

R₉ is selected from the group consisting of acetylene, lower alkyl, lower alkene, cyano, phenyl, substituted phenyl, heterocyclic aromatic, and combinations thereof, the substituted phenyl having the structure:



15 wherein:

X₁-X₆ taken separately are hydrogen, chlorine, fluorine, lower alkyl, carboxylic acid, sulfonic acid, -CH₂OH, or linking group.

2. The compound of **claim 1** wherein one of Y₁ and Y₂ is oxygen and the other is hydroxyl.

3. The compound of **claim 1** wherein:

5 X₁ is selected from the group consisting of carboxylic acid, sulfonic acid, and -CH₂OH;

X₂ and X₅ taken separately are selected from the group consisting of hydrogen, chlorine, fluorine, and lower alkyl; and

10 X₃ and X₄ taken separately are selected from the group consisting of hydrogen, chlorine, fluorine, lower alkyl, carboxylic acid, sulfonic acid, and linking group.

4. The compound of **claim 1** wherein X₂ and X₅ are chlorine.

5. The compound of **claim 1** wherein X₁ is carboxylic acid.

15

6. The compound of **claim 1** wherein one of X₃ or X₄ is linking group, the other being hydrogen.

7. The compound of **claim 1** wherein one of X₁ or X₅ is selected from the group
20 consisting of carboxylic acid, sulfonic acid, and -CH₂OH.

8. The compound of **claim 1** wherein one of R₁-R₃ is fluorine.

9. The compound of **claim 8** wherein R₃ is fluorine.

25

10. The compound of **claim 1** wherein:

one of Y₁ and Y₂ is oxygen and the other is hydroxyl;

R₁ is a chlorine;

R₃ is a fluorine;

R₂ and R₄-R₈ are hydrogen; and
 R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₅ are chlorine, and one of X₃
 and X₄ is carboxyl and the other is hydrogen.

5 11. The compound of **claim 1** wherein:
 one of Y₁ and Y₂ is oxygen and the other is hydroxyl;
 R₁ and R₃ are fluorine;
 R₂, and R₄-R₈ are hydrogen; and
 R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₅ are chlorine, and one of X₃
 10 and X₄ is carboxyl and the other is hydrogen.

 12. The compound of **claim 1** wherein:
 one of Y₁ and Y₂ is oxygen and the other is hydroxyl;
 R₁ is methoxy, R₂ is chlorine, R₃ is fluorine;
 15 R₄-R₈ are hydrogen; and
 R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₅ are chlorine, and one of X₃
 and X₄ is carboxyl and the other is hydrogen.

 13. The compound of **claim 1** wherein:
 20 one of Y₁ and Y₂ is oxygen and the other is hydroxyl;
 R₃ is fluorine; R₁, R₂, and R₄-R₈ are hydrogen; and
 R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₅ are chlorine, and one of X₃
 and X₄ is carboxyl and the other is hydrogen.

25 14. The compound of **claim 1** wherein:
 one of Y₁ and Y₂ is oxygen and the other is hydroxyl;
 R₁-R₈ are hydrogen; and
 R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₅ are chlorine, and one of X₃
 and X₄ is carboxyl and the other is hydrogen.

15. The compound of **claim 1** wherein:

one of Y₁ and Y₂ is oxygen and the other is hydroxyl;

R₁ is chlorine; R₂-R₈ are hydrogen; and

5 R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₃ are chlorine, and one of X₃ and X₄ is carboxyl and the other is hydrogen.

16. The compound of **claim 1** wherein:

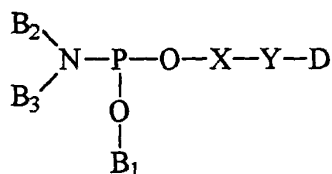
one of Y₁ and Y₂ is oxygen and the other is hydroxyl;

10 R₁ is methoxy; R₂ is chlorine;

R₃-R₈ are hydrogen; and

R₉ is substituted phenyl wherein X₁ is carboxyl, X₂ and X₃ are chlorine, and one of X₃ and X₄ is carboxyl and the other is hydrogen.

15 17. A phosphoramidite compound having the formula:



wherein:

X is a spacer arm;

Y is a linkage;

20 B₁ is a phosphite ester protecting group;

B₂, and B₃ taken separately are selected from the group consisting of lower alkyl, lower alkene, aryl, and cycloalkyl containing up to 10 carbon atoms; and

D is the dye compound of Claim 1;

wherein Y and D are linked through a linkage attached to dye D at one of positions

25 R₁-R₉.

18. The compound of **claim 17** wherein B₂ and B₃ taken together form an alkene chain containing up to 5 carbon atoms in the principle chain and a total of up to 10 carbon atoms with both terminal valence bonds of said chains being attached to the nitrogen atom; or B₂ and B₃ taken together with the nitrogen atom form a saturated nitrogen heterocycle which contains
 5 one or more heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur.

19. The compound of **claim 18** wherein:

B₁ is selected from the group consisting of methyl, β-cyanoethyl, or 4-nitrophenylethyl;

10 B₂ and B₃ taken separately are selected from the group consisting of isopropyl, t-butyl, isobutyl, and sec-butyl; and

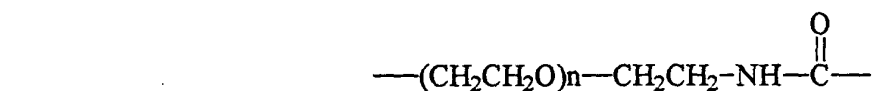
B₂ and B₃ taken together is morpholino.

20. The compound of **claim 17** wherein X and Y taken together is



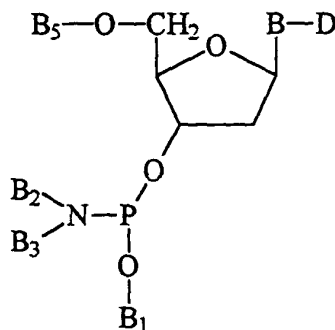
wherein n ranges from 2 to 10.

21. The compound of **claim 17** wherein X and Y taken together is



wherein n ranges from 2 to 10.

22. A phosphoramidite compound having the formula:



wherein:

B₁ is a phosphite ester protecting group;

B₂ and B₃ taken separately are selected from the group consisting of lower alkyl,
5 lower alkene, aryl, and cycloalkyl containing up to 10 carbon atoms;

B₅ is an acid-cleavable hydroxyl protecting group;

B is a nucleotide base; and

D is the dye compound of Claim 1;

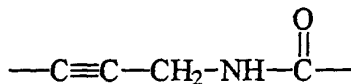
wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N⁹-
10 position of the purine or 7-deazapurine, and when B is pyrimidine, the sugar moiety is attached
at the N¹-position of the pyrimidine;

wherein B and D are linked through a linkage attached to D at one of positions R₁-R₉;

and

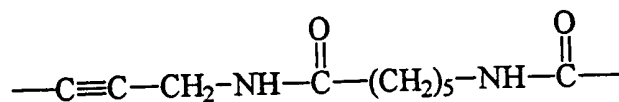
wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B is 7-
15 deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is
pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

23. The compound of **claim 22** wherein the linkage is

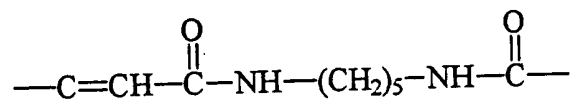


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24. The compound of **claim 22** wherein the linkage is



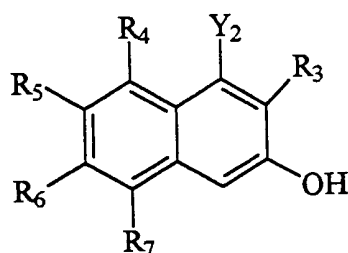
25. The compound of **claim 22** wherein the linkage is



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26. The compound of **claim 22** wherein B is selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

27. A compound having the formula:



10

wherein:

R_3 - R_7 taken separately are selected from the group consisting of hydrogen, fluorine, chlorine, lower alkyl, lower alkene, lower alkyne, sulfonate, amino, amido, nitrile, lower alkoxy, and linking group; and

15

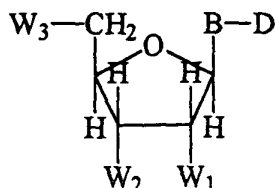
Y_2 is selected from the group consisting of hydroxyl and amine.

28. The compound of **claim 27** wherein R_3 is fluorine.

29. The compound of **claim 27** wherein Y_2 is hydroxyl.

20

30. A labeled nucleotide having the formula:

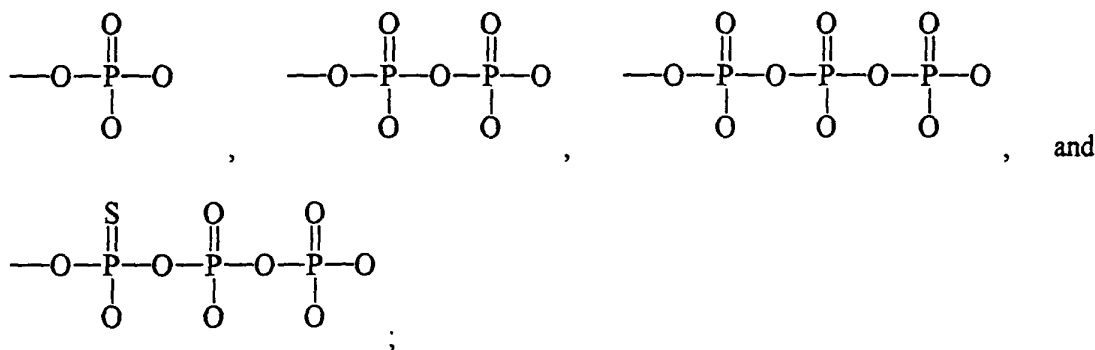


wherein:

B is a 7-deazapurine, purine, or pyrimidine nucleotide base;

W_1 and W_2 taken separately are selected from the group consisting of H and OH;

5 W_3 is selected from the group consisting of OH,



D is the dye compound of Claim 1;

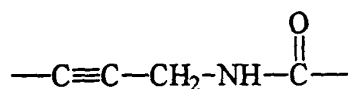
10 wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N^9 -position of the purine or deazapurine, and when B is pyrimidine, the sugar moiety is attached at the N^1 -position of the pyrimidine;

wherein the linkage linking B and D is attached to D at one of positions R_1 - R_9 ; and

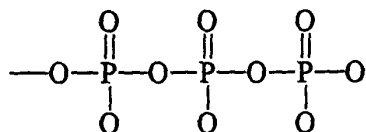
15 wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B is 7-deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

31. The labeled nucleotide of **claim 30** wherein B is selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

20 32. The labeled nucleotide of **claim 30** wherein the linkage is

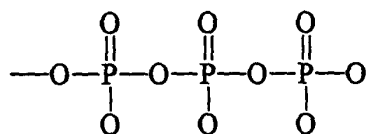


33. The labeled nucleotide of claim 30 wherein both W_1 and W_2 are H; and W_3 is

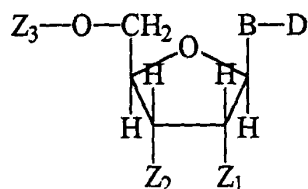


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34. The labeled nucleotide of **claim 30** wherein W₁ is H; W₂ is OH; and W₃ is



35. A labeled polynucleotide containing a nucleotide having the formula:



10

wherein:

B is a 7-deazapurine, purine, or pyrimidine nucleotide base;

Z_1 is selected from the group consisting of H and OH;

Z₂ is selected from the group consisting of H, OH, HPO₄, and Nuc, wherein Nuc

15 and the nucleoside are linked by a phosphodiester linkage, the linkage being attached to the 5'-position of Nuc;

Z₃ is selected from the group consisting of H, HPO₃, phosphate analogs, and Nuc, wherein Nuc and the nucleoside are linked by a phosphodiester linkage, the linkage being attached to the 3'-position of Nuc;

and, D is a dye compound of Claim 1;

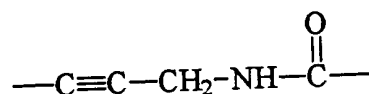
wherein when B is purine or 7-deazapurine, the sugar moiety is attached at the N⁹-position of the purine or deazapurine, and when B is pyrimidine, the sugar moiety is attached at the N¹-position of the pyrimidine;

5 wherein the linkage linking B and D is attached to D at one of positions R₁-R₉; and

wherein if B is a purine, the linkage is attached to the 8-position of the purine, if B is 7-deazapurine, the linkage is attached to the 7-position of the 7-deazapurine, and if B is pyrimidine, the linkage is attached to the 5-position of the pyrimidine.

10 36. The labeled polynucleotide of **claim 35** wherein B is selected from the group consisting of uracil, cytosine, deazaadenine, and deazaguanosine.

37. The labeled polynucleotide of **claim 35** wherein the linkage is



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38. A method of polynucleotide sequencing comprising the steps of:

forming a mixture of a first, a second, a third, and a forth class of polynucleotides such

that:

20 each polynucleotide in the first class includes a 3'-terminal dideoxyadenosine and is labeled with a first dye;

each polynucleotide in the second class includes a 3'-terminal dideoxycytidine and is labeled with a second dye;

each polynucleotide in the third class includes a 3'-terminal dideoxyguanosine and is labeled with a third dye; and

25 each polynucleotide in the forth class includes a 3'-terminal dideoxythymidine and is labeled with a forth dye;

wherein one of the first, second, third, or forth dyes is the asymmetric benzoxanthene dye of Claim 1;

the other of the dyes being spectrally resolvable from the asymmetric benzoxanthene dye and from each other;

5 electrophoretically separating the polynucleotides thereby forming bands of similarly sized polynucleotides;

illuminating the bands with an illumination beam capable of causing the dyes to fluoresce; and

10 identifying the classes of the polynucleotides in the bands by the fluorescence spectrum of the dyes.

39. The method of **claim 38** wherein the other of the dyes are selected from the group consisting of 6-carboxyfluorescein, 6-carboxy-4,7,2',7'-tetrachlorofluorescein, and 6-carboxy-4,7,2',4',5',7'-hexachlorofluorescein.

15

40. A method of fragment analysis comprising:

forming a labeled polynucleotide fragment, the fragment being labeled with the dye compound of Claim 1;

20 subjecting the labeled polynucleotide fragment to a size-dependent separation process; and

detecting the labeled polynucleotide fragment subsequent to the separation process.

41. The method of **claim 40** wherein the size-dependent separation process is electrophoresis and the detecting is by fluorescence.